

Targeting of surface alpha-enolase inhibits the invasiveness of pancreatic cancer cells

Supplementary Material

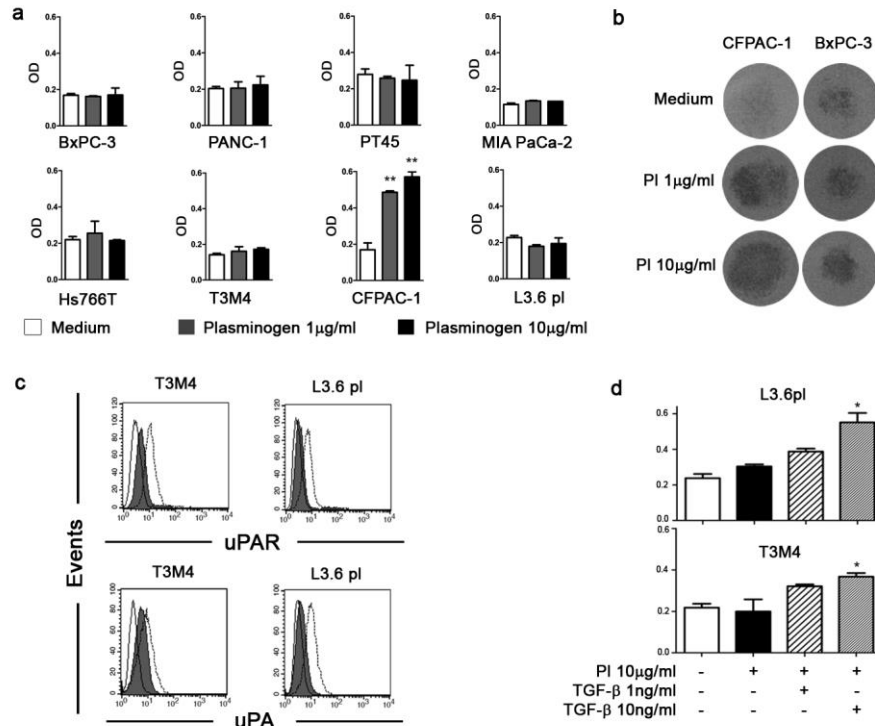


Figure. S1: Invasive potential of PDAC cell lines in presence of plasminogen. (a)

PDAC cells were placed on Matrigel coated transwell filters and plasminogen added in appropriate conditions (1 μ g/ml and 10 μ g/ml). After 48 hours, migrated cells were fixed, stained with crystal violet and dissolved. The eluates were read at the spectrophotometer at 570 nm wavelength. Results are expressed as mean \pm SEM of Optical Density units (OD) and conditions were in triplicate. CFPAC-1 invasion in response to plasminogen is significantly different compared to medium only condition. One representative of three independent experiments is shown. **(b)** A representative image of transwell membrane stained for each condition is reported for CFPAC-1 and BxPC-3 cells. **(c)** To evaluate uPA and uPAR overexpression after 24h of treatment with TGF- β (10ng/ml), flow-cytometry analysis was performed. PDAC cell line were incubated with anti-uPAR antibody for surface staining (solid histogram without TGF- β and dashed line after treatment with TGF-

β) or fixed with PFA, treated with Saponin and incubated with anti-uPA mAb (solid histogram without TGF- β and dashed line after treatment with TGF- β) for intracytoplasmic staining. Isotype matched control antibody was used as control (open histogram). One representative of three independent experiments is shown. **(d)** L3.6pl and T3M4 cells were placed on Matrigel coated transwell filters were added in appropriate conditions plasminogen (10 μ g/ml) and TGF- β (1ng/ml or 10ng/ml). Experiment was analyzed as described before. PDAC cells invasion in response to TGF- β (10ng/ml) is significantly different compared to plasminogen condition. One representative of three independent experiments is shown. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

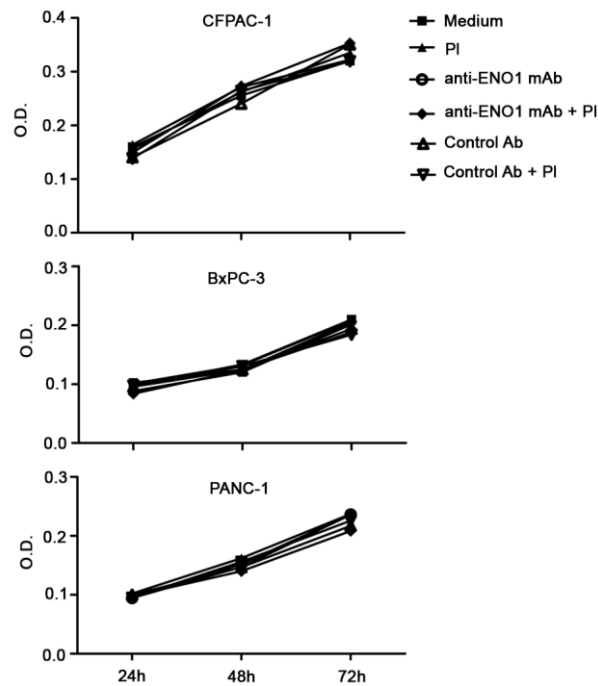


Figure. S2: Effect of anti-ENO1 mAb on proliferation. CFPAC-1, BxPC-3 and PANC-1 cells survival was assessed by MTT assay. Cells were starved and after 36h 2% FBS was added with or without plasminogen (10 μ g/ml), anti-ENO1 72/1 mAb (50 μ g/ml) or control Ab depending on the different conditions. MTT solution was added 24, 48 or 72 hrs after serum replenishment. O.D. values were measured at 570 nm.

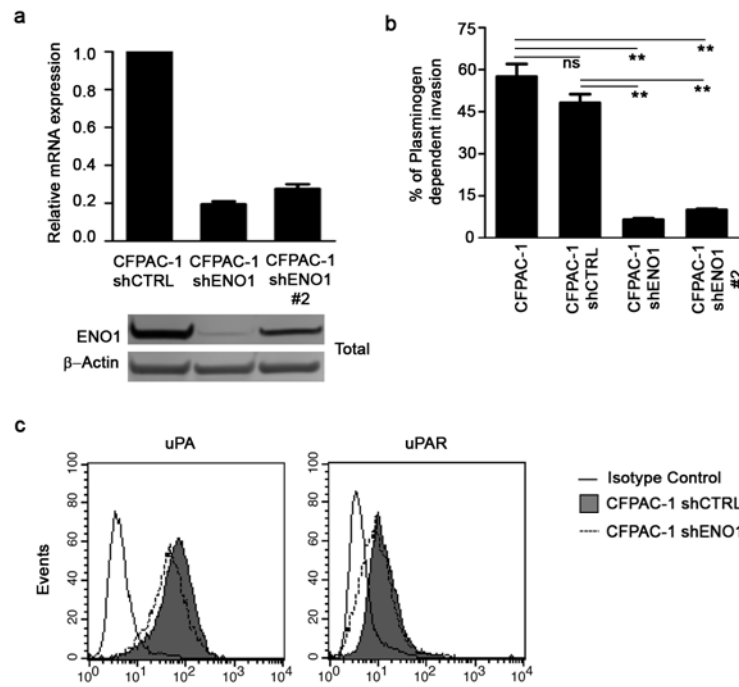


Figure. S3: Knockdown of ENO1 in CFPAC-1 cell lines. (a) To confirm the silencing of ENO1 at mRNA and protein level, Real-Time PCR (a upper panel) and Western Blot analysis was performed respectively (a lower panel). Results were normalized using β -Actin. CFPAC-1 shCTRL cell line was used as control. (b) CFPAC-1 parental or shCTRL shENO1 and shENO1#2 CFPAC-1 were placed on Matrigel-coated transwell filters and plasminogen (10 μ g/ml) were added in appropriate conditions. Results represent the percentage of plasminogen-dependent invasion calculated as: (OD of migrated cells in the presence of plasminogen / OD of cells migrated in the absence of plasminogen) x100. The different conditions were repeated in triplicate. (c) CFPAC-1 shENO1 and shCTRL cell lines were incubated with anti-uPA antibody (left panel), anti-uPAR (right panel) or isotype-matched control antibody and analyzed by flow-cytometry. One representative of three independent experiments is shown. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

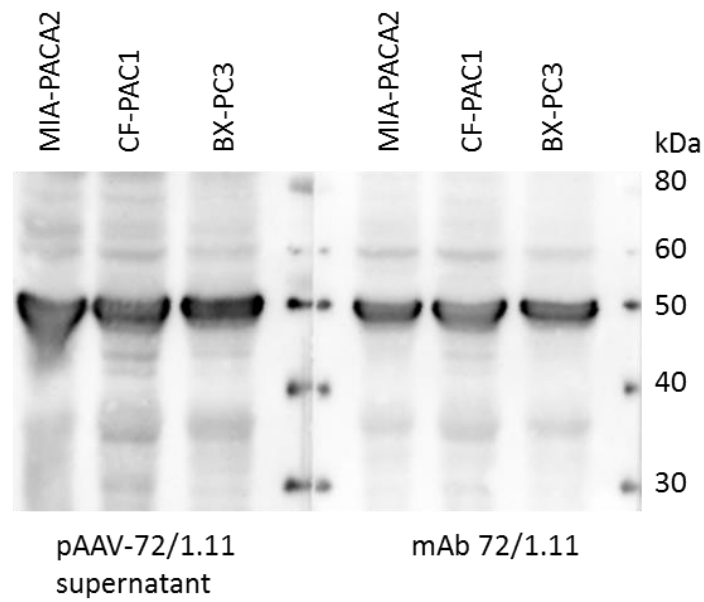


Figure. S4: Construction of the recombinant adeno-associated viral vector (AAVV) for the expression of complete anti-ENO1 mAb Western blotting of extracts from the indicated pancreatic cancer cell lines, probed with either the original mAb 72/1.11 or supernatant from HEK293 cells transfected with the pAAV-72/1.11 vector and revealed with HRP-conjugated anti-mouse IgG antibodies. The same 48 kDa band, corresponding to ENO1 is detected in all lanes.

Table S1: Plasminogen-related protein expression and invasion in PDAC cells

CELL LINE	uPA ^a	uPAR ^b	ENO1 ^c	INVASION ^d
<i>BxPC-3</i>	+	+	-	no
<i>PANC-1</i>	+	+	-	no
<i>PT45</i>	-	+	+	no
<i>MIA PaCa-2</i>	-	+	+	no
<i>Hs766T</i>	-	+	+	no
<i>T3M4</i>	+	+(*)	+	Yes(*)
<i>CFPAC-1</i>	+	+	+	Yes
<i>L3.6pl</i>	+(*)	+(*)	+	Yes(*)

LEGEND

uPA^a, uPAR^b and ENO1^c expression was determined by flow-cytometry as reported Fig.1. The results were arbitrarily assigned based on Δ MFI values.^d Invasion capability was determined by invasion transwell assay as reported in Fig. S1a.

(*) after TGF- β treatment as reported in Fig. S1 c-d.

Table S2: Primers used for mutagenesis and sequencing of ENO1

<i>Primers</i>	<i>Sequence</i>
ENO1 XhoI forward	5'-CCGCTCGAGTATGTCTATTCTCAAGATCCA-3'
ENO1 NotI reverse	5'-TAGCGGCCGCTTACTTGGCCAAGGG
EnoK420R ,K422R K434R forward	5'-GAAGAGGAGCTGGGCAGCAGGGCTAGGTTTGCCGGCAGGAAC-3'.
Eno K420R ,K422R K434R reverse	5'-GTTCTGCGGGCAAACCTAGCCCTGCTGCCCACGTCCTCTTC-3'.
ENO1 qPCR forward	5'-GCCTCCTGCTCAAAGTCAAC-3'
ENO1 qPCR reverse	5'-AACGATGAGACACCATGACG-3'
Beta Actin Forward	5'-CGCCGCCAGCTCASCATG-3'
Beta Actin Reverse	5'-CACGATGGAGGGGAAGACGG-3'
SEQ 1ENO	5'-TGTACGGTGGGAGGTCTATA-3'
SEQ 2ENO	5'-GATGGATGGAACAGAAAATA-3'
SEQ 3ENO	5'-TACACTGATAAGGTGGTCAT-3'
SEQ 4ENO	5'-GTCATGGTGTCTCATCGTTC-3'
SEQ 5ENO	5'-TTCCATCCATCTCGATCATCA-3'